Project Report: Protenomes of Permafrost Bateria

Project Investigators:	David Lubman , Michael Thomashow , James Tiedje

Project Progress

The goal of this project is to identify changes that occur in the proteomes of permafrost bacteria in response to low temperature, high osmolarity, and other conditions associated with the permafrost environment. One line of experimentation involves the development of a novel 2−dimensional liquid fractionation method as a replacement for two−dimensional gel electrophoresis. The method uses a pH column−based separation in the first dimension followed by separation of the proteins in each pH fraction using nonporous silica (NPS) reversed phase high−performance chromatography (HPLC). Using this method, we have been able to detect about 500 proteins in *Psychrobacter* 273−4. A comparison of cells grown at 22 versus 14°C has revealed that over 40 proteins are differentially expressed at these two temperatures, some being essentially qualitative changes. The identity of these polypeptides have been determined by using maxtrix−assisted laser desorption/ionization−time−of−flight (MALDI−TOF) mass spectrometry and matching the peptide mass maps against the deoxyribonucleic acid (DNA) sequence database that we have generated for *Psychrobacter* 273−4 in our genome sequencing project. Nearly a third of the proteins are conserved hypothetical polypeptides with unknown function, with most of the others being involved in translation or metabolism. A comparison of these results with the transcriptome results, suggests that posttranslational regulation may play a major role in temperature−regulated gene expression in *Psychrobacter* 273−4.

Proteome analysis has also been conduced with *Psychrobacter cryopegella* , a bacterium isolated from a briny water lens (−12 ° C) within the Siberian permafrost. *P. cryopegella* was found to grow at temperatures between −10 and 28 ° C and to remain physiologically active at −20 ° C. Two−dimensional gel analysis followed by liquid chromatography−double mass spectrometry was used to identify cold acclimation proteins (CAPs); i.e., proteins that were uniquely or preferentially expressed at temperatures below 0 ° C. Of the total 756 proteins that were detected, 24 were classified as CAPs. Of these, 20 were identified as open reading frames (ORF) within the genome of *Psychrobacter* 273−4. Three of the ORFs were annotated as conserved hypothetical proteins. Of the remaining ORFs, 11 had previously been identified as stress response

proteins in other organisms; these included superoxide dismutase, the ribosomal ribonucleic acid (rRNA)–binding general stress protein Ctc, aspartate–semialdehyde dehydrogenase, the periplasmic component of a TRAP–type transport system, and the protein chaperone ppiB. The identities of these CAPs suggested the importance of oxidative stress, increased energy needs, and compatible solutes for growth at subzero temperatures.

Highlights

- Proteins present only during growth at subzero temperatures were identified in *Psychrobacter cryopegella* and may be involved in relieving the stresses caused by living at low temperatures.
- Methodologies for reproducible, automated proteome mapping have been developed for Psychrobacter strains and used to identify changes in protein composition that occurs in response to temperature.
- Evidence suggests that cold–regulated gene expression in *Psychrobacter* 273–4 may involve posttranscriptional control mechanisms.

Roadmap Objectives

- ***Objective No. 5.1:*** Environment–dependent, molecular evolution in microorganisms
- ***Objective No. 5.3:*** Biochemical adaptation to extreme environments
- ***Objective No. 6.2:*** Adaptation and evolution of live beyond Earth